

Claims

1. A method for reducing seed shattering in a plant, preferably a *Brassicaceae* plant comprising the following steps:

5 (1) creating a population of transgenic lines of said plant, wherein said transgenic lines of said population exhibit variation in podshatter resistance, and wherein said population is obtainable by

(i) introducing a chimeric gene into cells of said plant, to create transgenic cells, said chimeric gene comprising the following operably linked DNA:

10 (a) a plant-expressible promoter;

(b) a DNA region which when transcribed yields a double-stranded RNA molecule capable of reducing the expression of a gene endogenous to said plant, preferably endogenous to said *Brassicaceae* plant, said gene being involved in the development of a dehiscence zone and valve margin of a pod of said plant, and said RNA molecule comprising a
15 first and second RNA region wherein

(i) said first RNA region comprises a nucleotide sequence of at least 19 consecutive nucleotides having about 94% sequence identity to the nucleotide sequence of said endogenous gene involved in the
20 development of a dehiscence zone and valve margin of said pod;

(ii) said second RNA region comprises a nucleotide sequence complementary to said 19 consecutive nucleotides of said first RNA region;

(iii) said first and second RNA region are capable of base-pairing to form a double stranded RNA molecule between at least said 19
25 consecutive nucleotides of said first and second region;

(c) optionally, a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of said plant;

wherein said chimeric gene, when expressed in cells of said plant, preferably said
30 *Brassicaceae* plant increases podshatter resistance compared to podshatter resistance in

an untransformed plant, preferably an untransformed *Brassicaceae* plant, while maintaining an agronomically relevant threshability of said pods of said plant;

- (ii) regenerating transgenic lines from said transgenic cells; and
 - (2) selecting a podshatter resistant plant from said population wherein said plant has
- 5 pods exhibiting reduced seed shattering.

2. The method of claim 1 wherein said plant expressible promoter is a relatively weak plant expressible promoter.

10 3. The method of claim 1, wherein said plant expressible promoter is an opine synthetase promoter from *Agrobacterium* spp., preferably a promoter selected from a nopaline synthetase promoter, an octopine synthetase promoter, a agrocinopine synthetase promoter or a mannopine synthetase promoter, or a dehiscence zone or valve margin selective promoter.

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4. The method of claim 2 or 3, wherein

- (i) said first RNA region comprises a nucleotide sequence of about 19 to about 500 consecutive nucleotides having a sequence similarity of about 90% to about 100% to the nucleotide sequence of said endogenous gene;
- 20 (ii) said second RNA region comprises a nucleotide sequence having about 90 to about 100% sequence similarity to the complement of the nucleotide sequence of said first RNA region; and
- (iii) said first and second RNA region are capable of forming a double stranded RNA region.

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5. The method of claim 1, wherein

- (i) said first RNA region comprises a sequence of about 50 to about 500 consecutive nucleotides having about 50% to about 88% sequence identity with said endogenous gene;

- (ii) said second RNA comprises a nucleotide sequence having about 90 to about 100% sequence similarity to the complement of the nucleotide sequence of said first RNA region; and
- (iii) said first and second RNA region are capable of forming a double stranded RNA region.
6. The method of claim 5, wherein said first RNA region comprises a sequence of about 200 to 300 consecutive nucleotides having about 65% to about 75% sequence identity with said endogenous gene.
7. The method of any of the preceeding claims, wherein said endogenous gene is a gene selected from the group of *INDEHISCENT* gene from *Arabidopsis thaliana*, *ALCATRAZ* gene from *Arabidopsis thaliana*, *SHATTERPROOF1* gene from *Arabidopsis thaliana*, *SHATTERPROOF2* gene from *Arabidopsis thaliana* or a homologous gene thereof present in said *Brassicaceae* plant.
8. The method of claim 7, wherein said endogenous gene comprises the nucleotide sequence of SEQ ID No 2.
9. The method of claim 7, wherein said endogenous gene comprises the nucleotide sequence of SEQ ID No 3.
10. The method of any one of claims 1 and 5 to 7, wherein
- (i) said *Brassicaceae* plant is oilseed rape;
- (ii) said first RNA region comprises a nucleotide sequence comprising at least 19 consecutive nucleotides from the nucleotide sequence of a second gene involved in the development of a dehiscence zone and valve margin of a pod, said second gene being endogenous to a *Brassicaceae* plant different from oilseed rape;
- (iii) said second RNA comprises a nucleotide sequence having about 90 to about 100% sequence similarity to the complement of the nucleotide sequence of said first RNA region; and

(iv) said first and second RNA region are capable of forming a double stranded RNA region.

11. The method of claim 10, wherein said first RNA region comprises at least about
5 50 to about 500 consecutive nucleotides of said second gene involved in the development of a dehiscence zone and valve margin of a pod.
12. The method of claim 10 or 11, wherein said second gene involved the development of a dehiscence zone and valve margin of a pod is a gene selected
10 from the group of *INDEHISCENT* gene from *Arabidopsis thaliana*, *ALCATRAZ* gene from *Arabidopsis thaliana*, *SHATTERPROOF1* gene from *Arabidopsis thaliana*, *SHATTERPROOF2* gene from *Arabidopsis thaliana* or a homologous gene thereof present in a *Brassicaceae* plant.
- 15 13. The method of claim 12, wherein said nucleotide sequence of said first RNA region is selected from a region of said gene involved in the development of a dehiscence zone and valve margin of a pod other than a MADS-box region, a K-region or a bHLH region.
- 20 14. The method of any one of claims 1 to 4, wherein said first RNA region comprises a nucleotide sequence of at least 19 consecutive nucleotides from the nucleotide sequence of SEQ ID No 2 or SEQ ID No 3.
- 25 15. The method of any one of claims 1 to 4, wherein said first RNA region comprises a nucleotide sequence of about 50 to about 200 consecutive nucleotides from the nucleotide sequence of SEQ ID No 2 or SEQ ID No 3.
- 30 16. The method of any one of claim 1 to 15, wherein said agronomically relevant threshability corresponds to a half life time of the pods in a Random Impact test between 10 and 60 seconds.

17. The method of claim 16, wherein said agronomically relevant threshability corresponds to a half life time of the pods in a Random Impact test between 40 and 60 seconds.

5 18. A method for reducing seed shattering in an oilseed rape plant comprising the following steps:

(1) creating a population of transgenic lines of said oilseed rape plant, wherein said transgenic lines of said population exhibit variation in podshatter resistance, and wherein said population is obtainable by

10 (i) introducing a chimeric gene into cells of said oilseed rape plant, to create transgenic cells, said chimeric gene comprising the following operably linked DNA:

(a) a plant-expressible promoter;

15 (b) a DNA region which when transcribed yields a double-stranded RNA molecule capable of reducing the expression of a gene endogenous to said oilseed rape plant, said gene being involved in the development of a dehiscence zone and valve margin of a pod of said oilseed rape plant, and said RNA molecule comprising a first and second RNA region wherein

20 (i) said first RNA region comprises a nucleotide sequence of at least 50 consecutive nucleotides having at least about 90% sequence identity to the nucleotide sequence of a gene from *Arabidopsis thaliana* involved in the development of a dehiscence zone and valve margin of said pod;

25 (ii) said second RNA region comprises a nucleotide sequence complementary to said 50 consecutive nucleotides of said first RNA region;

30 (iii) said first and second RNA region are capable of base-pairing to form a double stranded RNA molecule between at least said 50 consecutive nucleotides of said first and second region;

- (c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of said plant;
- (ii) regenerating transgenic lines from said transgenic cells; and
- (2) selecting a podshatter resistant plant from said population wherein said plant has pods exhibiting reduced seed shattering.
19. The method of claim 18; wherein said gene involved the development of a dehiscence zone and valve margin of a pod is a gene selected from the group of *INDEHISCENT* gene from *Arabidopsis thaliana*, *ALCATRAZ* gene from *Arabidopsis thaliana*, *SHATTERPROOF1* gene from *Arabidopsis thaliana*, *SHATTERPROOF2* gene from *Arabidopsis thaliana*.
20. The method of claim 18, wherein said gene comprises the nucleotide sequence of SEQ ID No 1, SEQ ID No 2, SEQ ID No 3, SEQ ID No 9, SEQ ID No 10 or SEQ ID No 11 or a part of at least 19 consecutive nucleotides thereof.
21. The method of any one of claims 18 to 20, wherein
- (i) said first RNA region comprises a nucleotide sequence of at least 100 consecutive nucleotides having at least about 90% sequence identity to the nucleotide sequence of a gene from *Arabidopsis thaliana* involved in the development of a dehiscence zone and valve margin of said pod;
- (ii) said second RNA region comprises a nucleotide sequence complementary to said 100 consecutive nucleotides of said first RNA region;
- (iii) said first and second RNA region are capable of base-pairing to form a double stranded RNA molecule between at least said 100 consecutive nucleotides of said first and second region.
22. A chimeric gene as described in any one of claims 1 to 21.

23. A cell of a *Brassicaceae* plant comprising the chimeric gene according to claim 22.
24. A *Brassicaceae* plant obtainable by the methods of any one of claims 1 to 22.
- 5 25. A *Brassicaceae* plant comprising a chimeric gene according to claim 22 stably integrated into the genome of its cell.
- 10 26. Progeny of the *Brassicaceae* plant according to claim 24 or 25 comprising a chimeric gene according to claim 22 stably integrated into the genome of its cells.
- 15 27. Seed from the plants of the *Brassicaceae* plants of any one of the claims 24 to 26, or 25 comprising a chimeric gene according to claim 22 stably integrated into the genome of its cells.
- 20 28. An agricultural method comprising
- (i) sowing seeds according to claim 27 or planting plants according to any one of claims 24 to 26 in a field;
 - (ii) growing said plants until the pods are mature;
 - (iii) harvesting seeds from said pods by threshing with a combine harvester.